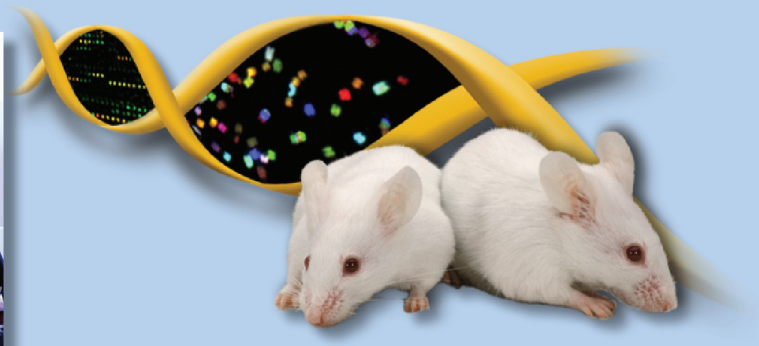
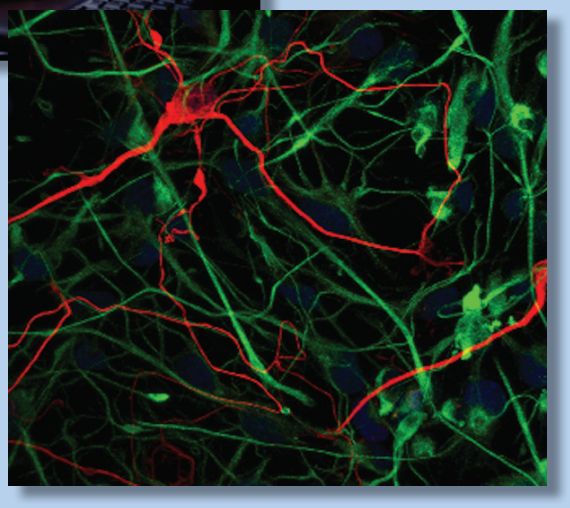
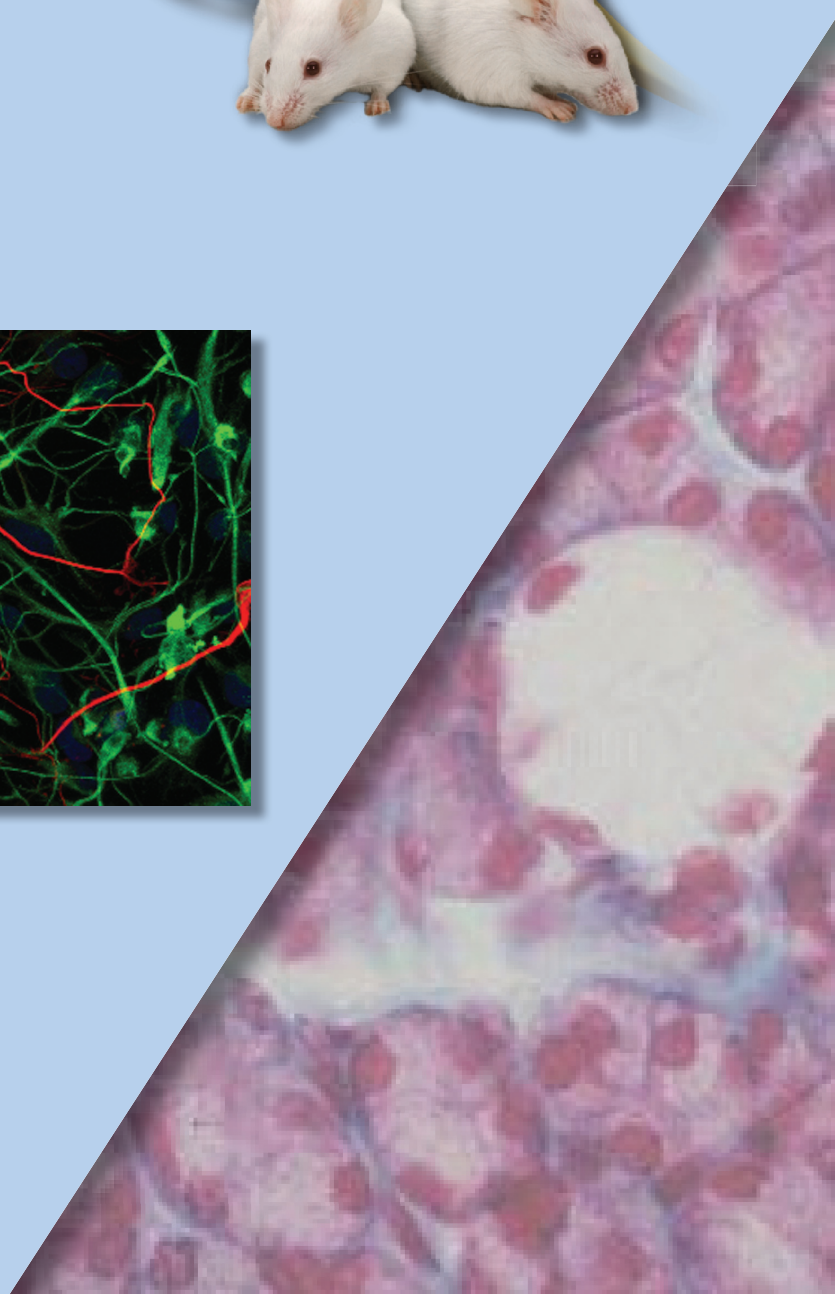
National Cancer Institute

NIH Comparative Biomedical Scientists Training Program Symposium

NIH Natcher Center,
Bethesda, Maryland
October 2–3, 2008







Sponsored by

The NCI Molecular Pathology Graduate Partnership Program

in collaboration with the NIAID, NIDDK, NHLBI, and the NINDS

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
National Institutes of Health

National Cancer Institute

CENTER FOR CANCER RESEARCH

Molecular Pathology Unit
Laboratory of Cancer Biology and Genetics
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October 2, 2008

Participants of the First Comparative Biomedical Scientists Training Program Symposium:

Welcome to this scientific symposium and training retreat, being held in the NIH Natcher Conference Center October 2 and 3, 2008, honoring the partnership established for interdisciplinary training of veterinarians in comparative pathology and biomedical research. As we commence this symposium, the training consortium is robust with scientifically and academically diverse institutional partners. Welcome to Michigan State University, North Carolina State University, University of Maryland, University of Illinois, and Purdue University from your hosts, The Intramural Research Divisions of The National Cancer Institute, The National Institute of Diabetes and Digestive and Kidney Diseases, The National Institute of Allergy and Infectious Diseases, The National Heart, Lung and Blood Institute, and The National Institute of Neurological Disorders and Stroke. Each member of the partnership contributes significantly to our mission; begun in 2003 and brought to fruition this summer with our first two graduates of the program, veterinarians Dave Caudell and Mark Hoenerhoff. Both have successfully achieved training as veterinary pathologists and been awarded the Doctor of Philosophy degree from the University of Maryland and Michigan State University, respectively, for original scholarship in cancer research accomplished with the university while in the Center for Cancer Research.

Our objectives in this first symposium include 1) highlighting research and research training being accomplished by veterinarians training in the NIH GPP interdisciplinary DVM/PhD training program, 2) informing our veterinary college-GPP university partners about NIH research, and for 3) developing interactive collaborations among partnership university faculty and NIH investigators. Toward this end, Thursday evening features a poster session for those of our veterinary pathologists-in-training who have formulated a Ph.D. dissertation problem. Earlier Thursday afternoon they will highlight their poster presentations from the platform to provide a preview for the evening's discussions. Our two program graduates will present their completed dissertation projects from the platform during Friday's session.

During 3 scientific sessions, we have invited a faculty member from each partnership university to present their research. To the degree possible, I sought to combine this presentation with one from an NIH investigator on a somewhat related topic. Finally, we are fortunate to gain perspectives from among each of the training consortium institutions with opening remarks provided by Michael Gottesman, M.D., our Deputy NIH Director for Intramural Research and Sharon Milgram, Ph.D. providing perspective as Director of the NIH Office of Training and Education. These presentations are followed by overviews of research and academic training programs being conducted by the various institute intramural programs and the partnership universities provided by our scientific directors and research deans, respectively. Additional meeting time is allotted to discuss how to facilitate further program development, including shared curricular content and scientific interactions. By our integrating veterinary and medical sciences, it is our intention for the symposium to inspire the forthcoming of mutual interests in bettering the public's health.

Thank you for participating in this first training program symposium.

R. Mark Simpson

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Acknowledgements

I would like to acknowledge and thank all the participants and sponsors of this symposium and the training program. Most noteworthy are those in-training, the NIH and university faculty investigators serving as mentors and graduate committee members, and the NIH staff members whose daily contributions make the program the valuable success it is. Many of these individuals are listed in the symposium participants list. The program's genesis would not have been possible without the early vision and support of several major contributors. Key among these is J. Carl Barrett, Ph.D., at the time our Scientific Director, Center for Cancer Research, NCI, Barbara J. Davis, V.M.D., Ph.D., at the time, Investigator, NIEHS, and Jonathan S. Wiest, Ph.D., Associate Director for Training and Education, Center for Cancer Research, NCI. I was encouraged in the undertaking of this effort by my former mentor, Tom Kindt, Ph.D., NIAID, who shares the vision of veterinarians making contributions as comparative biomedical scientists. Formative discussions and reviews of communications about the program were a part of the contributions made by L. Michelle Bennett, Ph.D., at the time, Associate Director for Science, CCR, NCI. We also received support from Mary DeLong, Ph.D., at the time, Director of Graduate Program Partnerships, NIH. A number of others from the GPP office were also supportive including Rick McGee, Ph.D., Caroline Duffy, M.S., and Pat Wagner, Ph.D.

In this partnership, our university partners provide critical elements necessary for the launch and growth of such a training initiative. Faculty from each university not only help to recruit, train, and mentor the trainees with proven curricular content developed over long personal tenures, but they also provide vision to the pathologists-in-training, for the unique benefits this kind of novel interdisciplinary training can provide. We are gratefully dependent upon and thankful for the support of faculty and administration at Michigan State University, North Carolina State University, University of Maryland, and University of Illinois for accomplishing the partnership agenda. To further our mission, we were most recently joined by Purdue University in this partnership.

As we continue to strive to add value to the public health research agenda in the United States, I am indebted to the sustained backing from two critical NIH sources of support. I thankfully acknowledge those institute Scientific Directors with whom I have the pleasure of working to develop the program. These include Robert Wiltrout, Ph.D., NCI, Tom Kindt, Ph.D, and presently Kathy Zoon, Ph.D, NIAID, Marvin Gershengorn, M.D., and presently Ira Levin, Ph.D., NIDDK, and Robert Balaban, Ph.D., NHLBI. Recently we were joined by Alan Koretsky, Ph.D., Scientific Director of NINDS. Their leadership motivates me to continue the excellence brought about by the many others contributing and participating in the program. Also, my colleagues within the Laboratory of Cancer Biology and Genetics, Center for Cancer Research including Shelley Hoover, Jennifer Edwards, Bih-Rong Wei, and John Hickerson are instrumental in operating the program and in providing training content to trainees. A special thank you also goes to Azalia Zandieh who, while she was a part of the Molecular Pathology Unit, helped us work through much of the early administrative structuring of program operations. Finally, I appreciatively acknowledge the support and encouragement of my mentor and lab chief Glenn Merlino, Ph.D. He very deservedly received award recognition as the NCI's 2008 Outstanding Mentor.

R. Mark Simpson

Member Institutions of the Molecular Pathology Graduate Partnership Program Consortium

National Institutes of Health Partners:

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University Partners:

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University of Illinois at Urbana-Champaign

Purdue University

NIH CBSTP Symposium
Agenda

The NCI Molecular Pathology Graduate Partnership Program in collaboration with the NIAID, NIDDK, NHLBI, and the NINDS announces the First

NIH Comparative Biomedical Scientist Training Program (CBSTP) Scientific Symposium and Retreat

October 2 & 3, 2008, The Natcher Center, NIH, Bethesda, MD

Thursday 10/2/2008 Symposium and Retreat NIH Natcher Center

12:00 pm – 1:30p	Meeting registration
1:30 pm – 4:30 pm	Scientific session **Natcher Room E1 and E2**
1:30 pm – 1:45 pm	Welcome and Recognition of University and NIH training mentors **Mark Simpson, D.V.M., Ph.D.**, Program Training Director, NCI
1:45 pm – 2:05 pm	The intramural research program **Michael Gottesman, M.D.**, Deputy Director for NIH Intramural Research, Office of the Director
2:05 pm – 2:15 pm	NIH Training **Sharon Milgram, Ph.D.**, Director, NIH Office of Intramural Training and Education, Office of the Director

Headline Event

2:15 pm – 2:45 pm	CBSTP molecular pathologist trainees' preview of individual training curricula – platform presentations of posters **Schantel Hayes, D.V.M.** **Yava Jones, D.V.M.** **Philip Martin, D.V.M.** **Tanasa Osborne, D.V.M.** **Heather Shive, D.V.M.** **Kevin Woolard, D.V.M.**
2:45 pm – 3:00 pm	*Break*

University and NIH investigators' scientific presentations (3:00 pm – 4:40 pm)

3:00 pm – 3:25 pm	**Yasmin Belkaid, Ph.D.**, NIAID *Role of induced and endogenous regulatory T cells in the control of parasitic infection*
3:25 pm – 3:50 pm	**Chang Kim, Ph.D.**, Purdue University *Migration of FoxP3+ regulatory T cells in a mouse model of Crohn's disease*

3:50 pm – 4:15 pm	**Katheryn Meek, D.V.M.**, Michigan State University *DNA-dependent protein kinase in mice and men: The means to justify the ends?*
4:15 pm – 4:40 pm	**Jeff Taubenberger, M.D., Ph.D.**, NIAID *Influenza Viruses: Past and Future Threats*
4:40 pm – 5:00 pm	Discussion of common collaborative interests, instructional methods round table
5:00 pm – 5:45 pm	Time to retire to the hotel for evening poster session and reception

Conference Hotel – Double Tree on Wisconsin Ave.

5:45 pm – 7:15 pm	Poster Session, all trainees, program faculty Refreshments will be served
7:15 pm	*Supper on own*

Friday 10/3/2008 Symposium and Retreat NIH Natcher Center

7:30 am – 8:30 am	Travel to campus and NIH Security Clearance to the Natcher Ctr
8:30 am – 8:45 am	Session Introductions **Mark Simpson, D.V.M., Ph.D.**, NCI

Presentation of Academic and Institutional Research and Education Programs

8:45 am – 9:15 am	**Robert Wiltrout, Ph.D.**, Director, NCI Center for Cancer Research
9:15 am – 10:20 am	**Ned Hahn, Ph.D.**, Associate Dean, University of Illinois **Kathy Zoon, Ph.D.**, NIAID Scientific Director **Siba Samal, BVSc, Ph.D.**, Associate Dean, University of Maryland
10:20 am – 10:35 am	*Break*
10:35 am – 12:25 am	**David Dorman, D.V.M., Ph.D.**, Associate Dean, North Carolina State University **Alan Koretsky, Ph.D.**, NINDS Scientific Director **Susan Ewart, D.V.M., Ph.D.**, Associate Dean, Michigan State U. **Robert Balaban, Ph.D.**, NHLBI Scientific Director **Harm HogenEsch, D.V.M., Ph.D.**, Dept. Chair, Purdue University
12:25 pm – 1:30 pm	Lunch, real-time interactive technology demonstration

CBSTP molecular pathologist graduate scientific presentations

Presentation of PhD Dissertation Research

1:30 pm – 1:45 pm	**David Caudell, D.V.M., Ph.D.**, Assistant Professor, Virginia Tech *Development of a mouse model for the t(10;11) (p13; q14) chromosomal translocation associated with acute leukemia in humans*
1:45 pm – 2:00 pm	**Mark Hoenerhoff, D.V.M., Ph.D.**, Staff Pathologist, NIEHS *BMI1 collaborates with HRAS to promote mammary tumorigenesis and metastasis*

University and NIH investigator scientific presentations

2:00 pm – 2:25 pm	**Tim Fan, D.V.M., Ph.D.**, University of Illinois *Fabricating bone-seeking nanoparticles for treating skeletal malignancy using canine osteosarcoma as a comparative model*
2:25 pm – 2:50 pm	**Lee Helman, M.D.**, NCI *(Title to be announced)*
2:50 pm – 3:05 pm	*Break*
3:05 pm – 3:30 pm	**Xiaoping Zhu, D.V.M., Ph.D.**, University of Maryland *MHC class I-related molecule meets IgG*
3:30 pm – 3:55 pm	**Natasha Olby, Vet.M.B., Ph.D.**, North Carolina State University *Spinal cord injury and rehabilitation medicine in dogs*
3:55 pm – 4: 20 pm	**Richard Youle, Ph.D.**, NINDS *Engineering apoptosis inhibitors for the treatment of spinal cord injury*
4:20 pm – 4:45 pm	Retreat Discussion of training program status and concepts for building collaborations Planning for upcoming symposium also known as closing remarks

Biographies and Research Abstracts
(Trainees with Dissertation Projects)

Schantel A. Hayes, D.V.M., Diplomate, The American College of Veterinary Pathologists

Dr. Hayes is a graduate fellow in the NCI Molecular Pathology Graduate Partnership Program in partnership with Michigan State University and the National Institute of Diabetes and Digestive and Kidney Diseases, from 2005 – present.

Dr. Hayes received her D.V.M. from Tuskegee University in 2004. Dr. Hayes pursued a residency in anatomic pathology in the Department of Pathobiology and Diagnostic Investigation at Michigan State University after graduation. In 2005, she was accepted into the NCI Molecular Pathology Graduate Partnership Program to continue graduate course work and training as a diagnostic pathologist at MSU. Following completion of her diagnostic training and course work, Dr. Hayes accomplished board certification in anatomic pathology by The American College of Veterinary Pathologists in 2007. She is now pursuing her Ph.D. dissertation research training within the National Institute of Diabetes and Digestive and Kidney Diseases, Bethesda, MD. Her research focuses on the transcriptional regulation of adipocyte differentiation in a laboratory headed by Elisabetta Muller, Ph.D., within the NIDDK Genetics of Development and Disease Branch. Members of her graduate guidance committee include Matti Kiupel D.V.M, Ph.D. (chair), Elisabetta Mueller, Ph.D., James Wagner, M.B.A., Ph.D., and Mark Simpson D.V.M, Ph.D.

MICHIGAN STATE UNIVERSITY

NIDDK
NATIONAL INSTITUTE OF DIABETES AND DIGESTIVE AND KIDNEY DISEASES

Characterization of Fat Specific NMC-1 Knockdown Mouse

Schantel A. Hayes[1,2], Sunitha Meruvu[1], and Elisabetta Mueller[1]

[1]Genetics and Development of Disease Branch, The National Institute of Diabetes and Digestive and Kidney Diseases, NIH, Bethesda, MD 20892; [2]Department of Pathobiology and Diagnostic Investigation, College of Veterinary Medicine, Michigan State University, East Lansing, MI

Mesenchymal stem cell differentiation during embryogenesis is a delicate balance of tightly controlled gene expression and transcriptional events driven by a series of transcription factors that lead to the development of muscle, bone, cartilage, and adipose tissue. PPARγ is considered to be the master regulator of the terminal differentiation phase of adipogenesis and functions in a transcriptional cascade that includes members of the CCAT/enhancer binding protein (C/EBP) family of basic helix loop helix transcription factors, and Kruppel like factor family. Although much is known about molecular regulation of the terminal differentiation phase, the transcriptional regulation of early adipogenesis (determination phase) is largely unknown. With this, it is important to study the transcriptional regulation of early adipogenesis and to research novel proteins that may regulate the transcription factors involved in terminal differentiation (PPARγ).

Recently, in our laboratory, a novel protein, NMC-1 was identified by candidate gene approach. NMC-1 is a transcription factor that belongs to the matrin 3 family of nuclear proteins and contains a splicing motif (RS motif), three RNA binding domains (RRM) and a zinc finger domain. NMC-1 was found to be highly expressed in mouse white adipose tissue and to co-activate PPARγ in both a ligand-dependent and ligand-independent manner. Overexpression of NMC-1 in 3T3-L1 cells was shown to promote adipogenesis and increases mRNA levels of genes involved in late stages of fat differentiation (PPARγ and Ap2). Inversely, knockdown of NMC-1 in 3T3-L1 leads to inhibition of adipogenesis and decreased levels of PPARγ and Ap2.

To validate the results obtained in vitro (3T3-L1 cells), a conditional knockdown mouse of NMC-1 was generated using Cre-LoxP induced RNA interference. The excision of the neocassette was implemented by crossing the NMC-1 knockdown mouse with transgenic mice that express Cre in the mouse white adipose tissue. The U6 promoter is then activated, leading to over 80% reduction of NMC-1 transcripts. Analysis of NMC-1 knockdown mice showed a decrease in body size and consistently and significantly a diminished weight compared to wild type mice at weaning onward. Furthermore, NMC-1 knockdown were lean with significant reductions in visceral and subcutaneous white adipose tissue and percentage of body fat when challenged on high fat diet. The white adipose tissue of NMC-1 knockdown mice appeared to contain smaller lipid droplets and often had regions that contained multilocular adipocytes with abundant eosinophilic cytoplasm. In conclusion, our preliminary in vitro and in vivo data suggest that NMC-1 is essential for white adipocyte development in postnatal life.

Yava L. Jones, D.V.M.

Dr. Jones is currently a graduate fellow in the NCI Molecular Pathology GPP in partnership with Michigan State University from 2004 – 2006 and 2007 – present.

Dr. Jones obtained a bachelors of arts from Talladega College in 1999 and her doctorate of veterinary medicine from Tuskegee University in 2003. She pursued an anatomic pathology residency at Michigan State University after graduation. In 2004, she was accepted into the NCI Molecular Pathology Graduate Partnership Program to continue her graduate course work and training as a diagnostic pathologist at MSU. Captain Jones interrupted her Cancer Research Training Award training at MSU from 2006-2007 while was deployed to Afghanistan in support of Operation Enduring Freedom with the United States Army Veterinary Corps. Following active duty, she returned to the program and is currently pursuing her Ph.D. dissertation research in the Cancer and Inflammation Program, Center for Cancer Research, at the National Cancer Institute located in Frederick, MD. Dr. Jones' current research topic involves studying the role of Tumor Necrosis Factor-α in the development and propagation of chemically induced colitis and colon cancer using mouse models. Her NIH principal investigator (PI) mentor is Giorgio Trinchieri, M.D. Her graduate committee members include Dr. Trinchieri, Matti Kiupel, Dr. Med. Vet, Ph.D., Diplomate, The American College of Veterinary Pathologists, Vilma Yuzbasiyan-Gurkan, Ph.D., Alison Bauer, D.V.M., and Mark Simpson, D.V.M., Ph.D., Diplomate, The American College of Veterinary Pathologists.

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The role of TNF-α in mouse models of colitis

Yava Jones[1,2], R. Salcedo[1], and G. Trinchieri[1]

[1]Cancer and Inflammation Program, National Cancer Institute, Frederick, MD; [2]Department of Pathobiology and Diagnostic Investigation, College of Veterinary Medicine, Michigan State University, East Lansing, MI

Inflammatory bowel disease (IBD) affects approximately 1.4 million people in the United States. IBD mainly consists of two disorders, ulcerative colitis (UC) and Crohn's disease (CD). CD is a transmural, granulomatous inflammatory condition that can affect any segment of the gastrointestinal tract. Irrespective of the subtype, IBD increases the risk for colorectal cancer. Tumor necrosis factor (TNF)-α has been implicated in the development of colitis and as a positive factor in the development of some forms of cancer, raising the possibility that TNF-α may play a tumor promoting role in colitis associated cancer. In fact, pharmacological blockade of TNF-α with monoclonal antibodies has shown great efficacy in many patients with IBD, however, side effects (reactivation of tuberculosis, development of autoimmune diseases, etc.) have occurred in small subset of individuals treated and the exact mechanism of action of these pharmacologics has not been elucidated. In light of the participation of epithelial cells and inflammatory cells in the tumorigenic process, the identification of the cell population that responds to TNF-α and that promotes tumor formation needs to be addressed.

Dextran sulfate sodium (DSS) is used as a "non-immune" model of colitis and is used primarily to study the innate immune mechanisms of colitis. In mice, blockage of TNF-α or it receptor has been shown to enhance acute DSS induced colitis and ameliorate or decrease DSS induced chronic colitis and colon cancer. However, the haptenating substance Trinitrobenzene sulfonic acid (TNBS) in ethanol (used to break the mucosal barrier), is a useful model of Crohn's disease in that is elicits a similar inflammatory, and cytokine (Th1/CD 4+ T cell mediated) and histopathologic (transmural, pancolonic, granulomatous) profile as the human disease. Mice with TNBS-induced colitis have improvement of clinical and histological disease when treated with antibodies to TNF- α. The role of the receptors for TNF (TNFR 1 and 2) have a differential role in TNBS colitis in that TNF-α signaling through TNFR1 appears to be protective, while TNF-α signaling through TNFR2 appears to promote pathology. The role of TNF and its contribution from specific cell types in TNBS induced colitis and colon cancer and acute DSS induced colitis will be explored in this project.

Philip L. Martin, M.S., D.V.M., Diplomate, The American College of Veterinary Pathologists

Dr. Martin is a graduate fellow in the NCI molecular pathology GPP in partnership with University of Maryland, 2005 – present.

Dr. Martin received his D.V.M. from Kansas State University in 2003. After completing the D.V.M. Dr. Martin went to the University of California, Davis for residency training in Anatomic Pathology in the Department of Veterinary Pathology. As an anatomic pathology resident Dr. Martin pursued specialty track training in the pathology of laboratory animals and undertook training in the UC Davis Comparative Pathology Laboratory and the Pathology Department of the California Regional Primate Center. In 2005 Dr. Martin began training in comparative pathology through the Graduate Partnership Program at the National Cancer Institute, Bethesda MD. The first year of the GPP program was spent completing graduate course work at the University of Maryland and in additional anatomic pathology training while working in the Comparative Molecular Pathology Unit with Dr. Mark Simpson. Dr. Martin accomplished board certification in Anatomic Pathology by The American College of Veterinary Pathologists in 2006. Dr. Martin is currently pursuing dissertation research in the NCI Cancer and Cell Biology Branch headed by Kathy Kelly, Ph.D. His Ph.D. dissertation research involves developing an in-vivo bioluminescent transgenic mouse model of prostate cancer metastasis for the purpose of investigating the molecular signaling mechanisms responsible for driving prostate cancer metastasis. Members of his graduate guidance committee include Siba Samal, B.V.Sc., Ph.D., Diplomate, The American College of Veterinary Microbiologists (chair), Xiaoping Zhu, D.V.M., Ph.D., Robert Dooling, Ph.D., Kathy Kelly, Ph.D., and Mark Simpson, D.V.M., Ph.D.

Development of an In-vivo Bioluminescent Mouse Model of Prostate Cancer Metastasis

Philip L. Martin[1,2], and Kathy Kelly[1]

[1]Cell and Cancer Biology Branch, National Cancer Institute, Bethesda, MD; [2]Department of Veterinary Medicine, University of Maryland, College Park, MD

The goal of this study is to establish a mouse model of prostate cancer metastasis that employs sensitive in-vivo bioluminescent imaging to monitor prostate cancer growth and metastasis. This model will then be used to test mechanistic hypotheses regarding which cell signaling pathways are involved in driving human prostate cancer metastasis.

The platform for this model is a transgenic mouse with prostate epithelial cell specific deletion of Pten and P53 as well as constituative luciferase expression. This mouse serves as the donor mouse for orthotopic transplantation of bioluminescent prostate tumor cells into immunocompromised recipient mice.

In order to create the donor mouse which has Pten and P53 deleted in the prostate epithelium, the cre-lox system of conditional gene deletion was employed. Transgenic mice with homozygous floxed Pten and P53 alleles were mated to a transgenic mouse expressing Cre recombinase under the control of the prostate specific probasin promoter. These mice, which have been previously described, develop prostatic intra-epithelial neoplasia (PIN) as early as 8 weeks of age with progression to adenocarcinoma and death due to urinary outflow obstruction in 100% of the mice between 3.5-8 months of age. . These male mice were then mated to female transgenic mice expressing the luciferase transgene under the control of the constituatively active B-actin promoter, to create the donor mice for orthotopic injections (Luciferase+PbCre+Pten-/- P53-/-).The adenocarcinoma in these donor mice is highly invasive with tumor cells frequently invading adjacent stroma, blood vessels, lymphatics, and nerves. Occasionally micrometastases can be identified in regional lymph nodes, and rarely circulating tumor cells can be identified in pulmonary capillaries. Older mice also develop spindle cell carcinomas.

The prostates of donor Luciferase+PbCre+Pten-/-P53-/- mice were harvested between 10-14 weeks of age, enzymatically digested into a single cell suspension and injected into the dorsal prostates of male athymic nude mice. In addition to these direct xenotransplantations, donor mouse cells were also cultured in a three dimensional matrix (matrigel) as protospheres for either short term (one generation:G1) or long term (nine generations:G9) culture before orthotopic injection into nude and Nod.Scid mice. Lymph node micrometastases from donor mice as well as cells harvested from first generation protospheres were expanded in 2D culture and the resulting cell lines were also used for orthotopic injections into nude and Nod.Scid mice. The recipient nude and Nod.Scid mice were then monitored regularly with the Xenogen in-vivo bioluminescence imaging system to assay for tumor growth and metastasis. Once tumor growth was confirmed with bioluminescent imaging and abdominal palpation, mice were euthanized and the orthotopic tumors were analyzed with histology and immunohistochemistry in order to determine their phenotypes.

The orthotopic tumors displayed the full range of histological patterns and immunophenotypes observed in the tumors of the donor mice. Tumors arising from donor

cells injected directly after harvest with no in-vitro culture (directly injected cells) formed adenocarcinomas, solid carcinomas, and occasionally adenosquamous carcinomas. These tumor cells had regions characteristic of PIN with basal (CK5), luminal (CK8) and transit amplifying cells (CK5+/CK8+) being present. Often these tumors displayed regions with only CK8+ carcinoma cells which closely resembled human prostate adenocarcinoma. Occasional tumor cells were synaptophysin+ indicating a neuroendocrine phenoptype. These tumors often demonstrated invasion of adjacent recipient mouse prostate, stroma, blood vessels, lymphatics, nerves, as well as lumbar musculature. Metastases were not detected in these mice.

Tumors arising from donor cells cultured as protospheres in matrigel for one week (G1 spheres) formed predominantly adenosquamous carcinomas with the adenocarcinoma regions containing CK5+, CK5+/CK8+, and CK8+ cells, and the squamous regions containing predominantly CK5+ cells with occasional CK5+/CK8+ cells and CK8+ cells. These tumors were also invasive and occasional regional lymph node metastases were observed, as well as rare lung metastasis.

The tumors arising from donor cells cultured for long term as protospheres (G9) formed tumors with two distinct phenotypes: adenocarcinoma with CK5+, CK5+/CK8+, and CK8+ cells, and a spindle cell carcinoma with heterogeneous and reduced CK8+ expression and lack of CK5 expression. The cell lines (grown in 2D standard culture conditions) arising from donor mouse lymph node micrometastases as well as those from G1 protospheres formed only spindle cell carcinomas with tumor cells expressing heterogeneous and reduced CK8 and no CK5. These spindle cell carcinomas arising from donor cells cultured in-vitro for long term were highly invasive with frequent metastasis to regional lymph nodes and rarely to the lung.

We have developed a mouse model of prostate cancer tumorigenesis and metastasis that utilizes bioluminescent imaging to monitor the growth and metastasis of Pten and P53 deleted prostate carcinoma cells. Bioluminescent imaging allowed the early detection of tumor growth and metastasis long before the tumors were grossly visible, and before histological detection was practical. A wide range of tumor phenotypes were observed in the donor luciferase+Pbcre+Pten-/-P53-/- mice, and these phenotypes were reproduced in the recipient mice. This likely indicates that tumors are arising from different subtypes of prostate epithelial cells. The orthotopic tumors arising from directly injected donor cells (with no in-vitro culture) were the most similar to human prostate carcinoma. Cell lines derived from these tumors will likely serve as the platform for future studies involving testing which cell signaling pathways are responsible for driving metastasis. This model has many advantages over currently used mouse prostate cancer models that employ either transgenic mice or xenotransplantation of human prostate cancer cells, and has great potential to help discover the cell signaling mechanisms that lead to prostate cancer tumorigenesis and metastasis.

Tanasa S. Osborne, D.V.M.

Dr. Osborne is a graduate fellow in the NCI molecular pathology GPP in partnership with University of Illinois at Urbana-Champaign, since 2006.

Dr. Osborne received her D.V.M. from Tuskegee University College of Veterinary Medicine in 2002. That same year, Dr. Osborne entered a combined residency/Ph.D. training program in Anatomic Pathology in the Department of Pathobiology at the University of Illinois at Urbana-Champaign. Upon completion of her residency in 2006 Dr. Osborne began graduate training in comparative pathology through the Graduate Partnership Program at the National Cancer Institute, Bethesda, MD. She is currently pursuing her Ph.D. dissertation research in metastasis biology in the Tumor and Metastasis Section, headed by Chand Khanna, D.V. M., Ph.D., Pediatric Oncology Branch. Her model system includes using a transplantable syngeneic mouse model characterized by orthotopic growth of osteosarcoma in BALB/c mice at appendicular sites with spontaneous metastasis to the lung. The title of her Ph.D. dissertation is "The role of eukaryotic initiation factor 4E (eIF4E) and an enabled translational machinery in osteosarcoma metastasis". Members of her graduate guidance committee include Wanda M. Haschek-Hock, B.V.Sc., Ph.D., Diplomate, The American College of Veterinary Pathologists (Chair), Chand Khanna, D.V.M., Ph.D., Diplomate, The American College of Veterinary Internal Medicine, Matthew A. Wallig, D.V.M., Ph.D, Diplomate, The American College of Veterinary Pathologists, Lois L. Hoyer, Ph.D., and Timothy M. Fan, D.V.M., Ph.D., Diplomate, The American College of Veterinary Internal Medicine.

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Expression of Eukaryotic Initiation Factor 4E (eIF4E) in Osteosarcoma

Tanasa Osborne[1,3], Ling Ren[1], Stephen Hewitt[2], and Chand Khanna[1]

[1]Tumor and Metastasis Biology Section, Pediatric Oncology Branch, National Cancer Institute, Bethesda, MD; [2]Tissue Array Research Program (TARP) Laboratory of Pathology, National Cancer Institute, Bethesda, MD; 3Department of Pathobiology, University of Illinois at Urbana-Champaign, Urbana, IL

The most significant problem for cancer patients is the dissemination of cancer cells and the formation of metastatic disease. Emblematic of the problem is the clinical progression seen in most patients with osteosarcoma, where metastasis to the lung is the most common cause of death. Cancer cells are believed to efficiently regulate protein translation at specific times and locations in a cell in response to changes in their environment. Within translation initiation the abundance and activation of the mRNA cap-binding phosphoprotein, eukaryotic initiation factor 4E (eIF4E) is considered to be both rate and process limiting. Using a comparative approach based on murine, canine, and human osteosarcoma cell lines and tissues we have begun to test the hypothesis that the metastatic success of osteosarcoma cells is linked to enabled protein translational machinery, defined by the expression and activity of eIF4E. Western analysis confirmed eIF4E expression in highly metastatic murine (K7M2) and human (143B and HOS-MNNG) osteosarcoma cells. Interestingly, lower expression was seen in clonally related and less metastatic variants of these cell lines (murine K12 and human HOS cells). Similar to murine and human cells, canine osteosarcoma cell lines were found to differentially express eIF4E. Semi-quantitative immunohistochemistry of multi-sample canine and human osteosarcoma tissue microarrays demonstrated that eIF4E is expressed in canine and human primary tumors and metastatic lesions. These initial data show that eIF4E is expressed in murine, canine, and human osteosarcoma cells and tissues. We seek to determine if eIF4E can be exploited as a novel therapeutic target in this commonly fatal pediatric cancer. We propose to modulate eIF4E expression and in murine, canine, and human osteosarcoma cell lines using various overexpression and knockdown techniques to define the role of eIF4E at various steps of the metastatic cascade *in vitro* and *in vivo*.

Heather R. Shive, D.V.M., Diplomate, The American College of Veterinary Pathologists

Dr. Shive is currently a graduate fellow in the NCI molecular pathology GPP in partnership with the University of Maryland from 2006 to present.

Dr. Shive received her D.V.M from North Carolina State University, College of Veterinary Medicine in 2004. Following graduation, she joined the residency program in anatomic pathology in the Department of Pathobiology at Texas A&M University, College of Veterinary Medicine. She trained for two years as a resident at TAMU, and was accepted as a graduate fellow in molecular pathology through the Graduate Partnership Program at the National Cancer Institute in 2006. Dr. Shive was concurrently accepted into the graduate program at the University of Maryland through the GPP. While pursuing graduate training at the NCI, she achieved board certification in Anatomic Pathology by the American College of Veterinary Pathologists in 2007. Dr. Shive is currently developing her dissertation research utilizing the zebrafish as a cancer model in the research laboratory of Dennis Hickstein, M.D., in the Experimental Transplantation and Immunology Branch at the NCI. The research studies are designed to generate a zebrafish model of ovarian cancer that recapitulates the human disease, and to use this model to investigate the individual and collaborative effects of three known risk factors for ovarian cancer: BRCA2 mutation, age, and reproductive history. Members of her graduate guidance committee include Siba Samal, BVSc, Ph.D., Diplomate, The American College of Veterinary Microbiologists (chair), Xiaoping Zhu, D.V.M, Ph.D., Paul Liu, M.D., Ph.D., Dennis Hickstein, M.D., and Mark Simpson, D.V.M, Ph.D., Diplomate, The American College of Veterinary Pathologists.

Modeling Ovarian Cancer in the Zebrafish

Heather Shive[1,3], West R[1], Bishop K[2], Sood R[2], and Hickstein D[1]

[1]Experimental Transplantation and Immunology Branch, National Cancer Institute; [2]Zebrafish Core facility, National Human Genome Research Institute, National Institutes of Health, Bethesda, MD; [3]Department of Veterinary Medicine, University of Maryland, College Park, MD

Our study aims to generate an animal model of ovarian cancer using the zebrafish (*Danio rerio*). Ovarian cancer is the fifth most common cause of cancer death in women in the United States, and the majority of the cases are diagnosed in an advanced stage with a correspondingly dramatic decrease in survival. Despite the importance of ovarian cancer as a cause of cancer-related morbidity and mortality in women, current investigations of disease pathogenesis and new therapeutic approaches are limited by the lack of an appropriate animal model of the disease.

In humans, a family history of breast or ovarian cancer represents one of the strongest predictors for ovarian cancer; approximately 90% of hereditary cases of ovarian cancer are attributable to mutations in the breast cancer associated genes 1 and 2 (BRCA1 and BRCA2). The majority of known deleterious BRCA2 mutations are predicted to result in premature protein truncation. In particular, mutations in exon 11 of the human BRCA2 gene (the 'Ovarian Cancer Cluster Region', or OCCR) confer an increased ovarian:breast cancer ratio in affected families. Other factors that increase the risk for developing ovarian cancer, independent of family history, are age and nulliparity.

Murine models of BRCA1 and BRCA2 mutation generated by gene targeting in embryonic stem cells do not exhibit the propensity for ovarian tumors that is observed in humans with mutations in these genes. This may be related in part to the large deletions in BRCA1 and BRCA2 induced by gene targeting in mouse models, or to factors intrinsic to the murine system.

These studies are designed to generate a zebrafish model of ovarian cancer that recapitulates the human disease by establishing zebrafish lines with BRCA2 mutations that are similar to mutations detected in women with hereditary breast and ovarian cancer syndromes. This model will be used to investigate the individual and collaborative effects of risk factors for ovarian cancer including BRCA2 mutation, age, reproductive history, and additional genetic events.

In the course of these studies, we analyzed the characteristics of the zebrafish ovarian surface epithelium (OSE) with histologic and immunohistochemical techniques and compared these results to the histologic and immunohistochemical characteristics of the OSE in ovarian tissue from humans and other species. These techniques demonstrate that the OSE from zebrafish exhibits similar immunohistochemical properties to other species, despite certain morphologic differences in the OSE among these species.

BRCA2 expression pattern and distribution in embryonic, larval, immature, and adult stages of zebrafish by in situ hybridization demonstrates expression of BRCA2 during embryonic and larval stages, particularly in the developing eye and central nervous system. These patterns of BRCA2 expression in zebrafish embryos are similar to those described in mouse embryos. BRCA2 is expressed most strongly in the ovary in immature and adult zebrafish.

To develop zebrafish lines with germline BRCA2 mutations, we screened an ethylnitrosourea (ENU)-mutagenized zebrafish library for mutations in zebrafish BRCA2 exon 11. Chemical mutagenesis through exposure to ENU permits the establishment of libraries of randomly mutagenized zebrafish that may be screened for mutations in specific genes of interest. Frozen sperm from mutagenized males allows lines harboring the mutation of interest to be rescued by in vitro fertilization. We have identified and successfully recovered a line of zebrafish with a nonsense mutation in zebrafish BRCA2 that is predicted to result in premature truncation of the BRCA2 protein. Zebrafish that are heterozygous for the Q658X mutation have now been generated and will be utilized for studies of BRCA2-associated cancer. These studies include the influence of age, ovulatory and reproductive history, and the influence of additional genetic mutations.

Establishing an ovarian cancer model in a genetically tractable model such as the zebrafish provides a unique opportunity to investigate and identify contributory factors in ovarian carcinogenesis. This model also represents a potential in vivo system for assessing novel therapeutic interventions for human ovarian cancer.

Kevin D. Woolard, D.V.M.

Dr. Woolard is a graduate scholar in the NCI Molecular Pathology GPP in partnership with the North Carolina State University from 2003 - present.

After graduating from veterinary school at North Carolina State in 2003, Kevin stayed at the university, entered the molecular pathology GPP as a Cancer Research Training Fellow and undertook graduate course work and training in anatomic pathology. During his diagnostic pathology training he formed an interest in neuro-pathology and in neural stem cell biology. These interests led to his pursuit in the establishing the domestic dog as a model for glioma tumor stem cell biology. His Ph.D. dissertation research in spontaneous canine glioma brain tumors focuses on the comparative biology of the tumor initiating cells. He is making exciting comparisons to human glioma tumor stem cells, as well as physiologic neural stem cells simultaneously isolated from canine patients. By discriminating genetic and cellular mechanistic differences new knowledge about neural and cancer stem cell development is being revealed. Kevin is a member of the Neuro-Oncology Branch, headed by Howard A. Fine, M.D. Other members of his graduate committee include John Cullen, V.M.D., Ph.D. Diplomate, The American College of Veterinary Pathologists (chair), Matthew Breen, Ph.D., Dave Malarkey, D.V.M., Ph.D., Diplomate, The American College of Veterinary Pathologists, and Mark Simpson D.V.M., Ph.D., Diplomate, The American College of Veterinary Pathologists.

Characterization of Canine Glioma Derived Tumor Stem Cells

Kevin Woolard[1,2,3], M. Totonchy[1], A. Li[1], M. Breen[4], R. Thomas[4], M. Beederman[1], E. Clark[1], M. Simpson[2], J. Lee[1], and H. Fine[1]

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While primary central nervous system tumors account for only 1.5% of new cancer cases per year, they represent the third leading cause of cancer-related death in men, and fourth leading cause of cancer-related death in women under the age of 54. The radioresistance and invasive growth of high-grade glioma tumors account in part for this high mortality rate, with grade IV glioblastoma tumors exhibiting a median survival time of only 11 months. The cancer stem cell hypothesis has widely been applied towards glioma tumors, with the belief that the identification and characterization of this small subset of tumor cells responsible for tumor propagation will improve patient survival and introduce novel therapeutic options. However, many fundamental questions regarding glioma tumor stem cell biology still remain unanswered, including the principle question of the relationship between glioma tumor stem cells and physiologic neural progenitor cells.

The domestic dog (*Canis familiaris*) is the only non-human species which readily develops spontaneous glioma brain tumors. These tumors exhibit a striking similarity not only in incidence, but also in biologic behavior, recapitulating every histologic grade, or sub-type identified in human neuropathology. However, comparative analysis of canine and human glioma tumors has been limited to superficial examinations to date. Here, we report the establishment of the first canine glioma-derived tumor stem cells from both a high-grade (grade III/IV on WHO grading scheme) mixed anaplastic glioma, and from a grade II astrocytoma. These tumor stem cells express many of the markers associated with human glioma tumor stem cells, including markers associated with maintenance of a stem-cell state (sox2, nestin) as well as multiple markers of differentiation (beta III-tubulin, GFAP, O4). Tumor stem cells derived from the high-grade glioma tumor efficiently form highly invasive, serially transplantable orthotopic xenograft tumors in SCID mice. In contrast, tumor stem cells from the grade II astrocytoma tumor exhibit low tumor uptake and a less invasive phenotype.

In an effort to compare these tumor stem cells to physiologic neural progenitor cells, samples from each patient were obtained from the mammalian neural stem cell niches-the olfactory bulb, subventricular zone, and the dentate gyrus of the hippocampus. These syngeneic physiologic neural progenitor cells express similar markers to both human and canine glioma tumor stem cells, exhibit similar growth characteristics in vitro, but do not produce tumors in SCID mice when implanted orthotopically. By comparing the gene expression profiles of the tumor stem cells to the patient-matched, syngeneic physiologic neural progenitor cells, we can for the first time examine the relationship of these cells within a single species. While it is believed that glioma tumor stem cells and these neural progenitor cells share common, key pathways involved in self-renewal and proliferation, the lack of widely available human progenitors has meant that prior investigations utilize murine

neural progenitor cells. The dog is the only species in which physiologic neural progenitors may be readily analyzed alongside syngeneic glioma tumor stem cells. By comparing the low and high-grade tumor stem cells to these neural progenitor cells, pathways of tumorigenesis, invasion, and blockade of differentiation may be revealed.

To examine the low and high-grade canine tumor stem cells for analogous chromosomal gains or losses, genomic DNA was hybridized using a custom bacterial artificial chromosome (BAC) array. These data indicate that the tumor stem cell population possesses the same genotype as the parental bulk tumor. Regions of chromosomal amplification or loss will be verified by fluorescent *in situ* hybridization (FISH) using single locus probes (SLPs) and then compared to common genes associated with human gliomagenesis. The canine diploid genome contains 78 chromosomes compared to the human 46, meaning that syntenic regions of human genes are widely dispersed across the canine genome. Thus, the array based CGH between the glioma tumor stem cells and physiologic neural progenitor cells not only serves to validate canine glioma chromosomal gains or losses in relation to those documented in human glioma, but may also highlight key regions within the human gene responsible for gain or loss of function.

We have reported the first characterization of canine glioma tumor stem cells. Canine glioma tumor stem cells exhibit similar in vitro growth characteristics, differentiation potential, and similar in vitro markers to human glioma tumor stem cells. Importantly, the study of spontaneous canine glioma tumors allows direct comparison of syngeneic physiologic neural progenitor cells to tumor stem cells. By comparing gene expression profiles of low and high-grade glioma tumor stem cells against patient-matched physiologic neural progenitor cells, we hope to demonstrate that low-grade tumor stem cells more closely resemble neural progenitor cells, while the simultaneous examination of high-grade tumor stem cells will reveal important pathways involved in gliomagenesis.

Biographies
(Trainees on University Campuses)

A. Sally Davis, D.V.M

Dr. Davis is currently a graduate scholar in the NCI Molecular Pathology GPP in partnership with North Carolina State University and the National Institute of Allergy and Infectious Diseases, from 2007 - present.

Dr. Davis received her D.V.M. from North Carolina State University in May, 2007. She is currently in her second-year of training as a graduate scholar in comparative pathology at the NCSU College of Veterinary Medicine, where she is taking graduate course work and training in diagnostic pathology. Her research interests are in emerging infectious diseases. Her academic advisors are John Cullen, V.M.D., Ph.D., Diplomate, The American College of Veterinary Pathologists, Keith Linder, D.V.M., Ph.D., Diplomate, The American College of Veterinary Pathologists, and Mac Law, D.V.M., Ph.D., Diplomate, The American College of Veterinary Pathologists.

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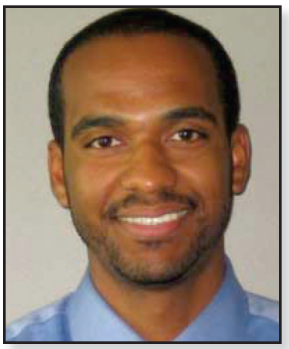

Ian N. Moore, D.V.M

Dr. Moore is a graduate fellow in the NCI molecular pathology GPP in partnership with Michigan State University, 2007-current.

Dr. Moore received his D.V.M. from Tuskegee University School of Veterinary Medicine in 2006, and following graduation, entered a 3-year anatomic pathology residency at Michigan State University's Diagnostic Center for Population and Animal Health. After completing one year of pathology residency Dr. Moore became an NCI Cancer Research Training Fellow as part of the Molecular Pathology GPP. Dr. Moore expects to complete all of the required didactic graduate course work required for his Ph.D. as well as his diagnostic pathology experience this 2008-2009 academic year. He intends to undertake his Ph.D. research training in the Center for Cancer Research, and seek certification by The American College of Veterinary Pathologists. Some of Dr. Moore's research interests include mechanisms of tumor metastasis and animal cancer models. His academic program advisors include Mattie Kiupel, Dr. Med. Vet., Ph.D., Diplomate, The American College of Veterinary Pathologists, Scott Fitzgerald, D.V.M., Ph.D., Diplomate, The American College of Veterinary Pathologists, Thomas Mullaney, D.V.M., Ph.D., Diplomate, The American College of Veterinary Pathologists, and Kurt Williams, D.V.M., Ph.D., Diplomate, The American College of Veterinary Pathologists.

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Heather S. Tillman, D.V.M

Dr. Tillman is currently a graduate scholar in the NCI Molecular Pathology GPP in partnership with the Michigan State University, beginning 2008.

Dr. Tillman received her D.V.M. from the University of Georgia in May, 2008. She is currently in her first-year of training as a graduate scholar in comparative pathology at the MSU Diagnostic Center for Population and Animal Health, where she is taking graduate course work and training in diagnostic pathology. Her research interests are in cancer biology, proteomics, and the development of animal models of human disease. Her academic advisor is Matti Kiupel, Dr. Med. Vet., Ph.D., Diplomate, The American College of Veterinary Pathologists.

Leah Zadrozny, D.V.M

Dr. Zadrozny is currently a graduate scholar in the NCI Molecular Pathology GPP in partnership with North Carolina State University and the National Heart, Lung, and Blood Institute, beginning 2008.

Dr. Zadrozny received her D.V.M. from North Carolina State University in May, 2008. She is currently in her first-year of training as a graduate scholar in comparative pathology at the NCSU College of Veterinary Medicine, where she is taking graduate course work and training in diagnostic pathology. Her research interests are in modeling the functional pathophysiology of cardiovascular disease with her Ph.D. dissertation studies planned in the NHLBI Laboratory of Cardiac Energetics headed by Robert Balaban, Ph.D. Her academic advisors are John Cullen, V.M.D., Ph.D., Diplomate, The American College of Veterinary Pathologists, Keith Linder, D.V.M., Ph.D., Diplomate, The American College of Veterinary Pathologists, and Mac Law, D.V.M., Ph.D., Diplomate, The American College of Veterinary Pathologists.

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Department of Health and Human Services • National Institutes of Health
National Heart Lung and Blood Institute
People Science Health

Biographies and Research Abstracts
(Program Graduates)

David Caudell, D.V.M., Ph.D.

Dr. Caudell was a graduate fellow in the NCI molecular pathology GPP in partnership with University of Maryland from 2004 – 2008.

Dr. Caudell received his D.V.M. from Virginia Tech in 2000. In 2003, Dr. Caudell completed his residency in Anatomic Pathology in the Department of Pathobiology at Oklahoma State University. Following completion of his residency, Dr. Caudell pursued graduate training in comparative molecular pathology through the Graduate Partnership Program at the National Cancer Institute, Bethesda, MD. He completed his dissertation research in mouse models of human leukemia in the Genetics Branch, Leukemogenesis Section headed by Peter D. Aplan, M.D. Dr. Caudell defended his dissertation research and received his Ph.D. degree from the University of Maryland in June 2008. The title of his Ph.D. dissertation is "Development of a mouse model for the t(10;11)(p13;q14) chromosomal translocation associated with acute leukemia in humans". Members of his graduate committee included Siba K. Samal, B.V.Sc. Ph.D., Diplomate, The American College of Veterinary Microbiologists (Chair), Peter D. Aplan, M.D. (Co-Chair), Nathanial Tablante, D.V.M.,M.S., Diplomate, The American College of Poultry Veterinarians, R. Mark Simpson, D.V.M., Ph.D., Diplomate, The American College of Veterinary Pathologists, Iqbal Hamza, Ph.D. and Nickolas Zimmerman, Ph.D. Dr. Caudell has accepted appointment as a tenure track Assistant Professor in Pathology, in the Department of Pathobiology and Biomedical Sciences at Virginia Tech, Blacksburg, VA.

Retroviral Insertional Mutagenesis Identifies collaborating genes that Accelerate Acute leukemia in CALM-AF10 Transgenic mice

David Caudell[1,6], Zhenhua Zhang[1], Yang Jo Chung[1], Rachel M. Pierce[2], David P. Harper[3], Rachel L. Novak[1], Christopher Slape[4], Linda Wolff[5], and Peter D. Aplan[1]

[1]Genetics Branch, Center for Cancer Research, NCI, NIH, Bethesda, MD; [2]Cell and Cancer Biology Branch, NCI, NIH, Bethesda, MD; [3]Department of Pediatrics, Uniformed Services University of the Health Sciences, Bethesda, MD; [4]Bone Marrow Research Laboratory, Royal Melbourne Hospital, Parkville, Australia; [5]Laboratory of Cellular Oncology, NCI, NIH, Bethesda, MD; [6]University of Maryland, College Park, MD

Leukemia is a hematological malignancy characterized by a predominance of immature hematopoietic or lymphoid precursor cells (acute leukemia) or an expansion of mature marrow elements (chronic leukemia). Signs and symptoms seen in acute leukemia are attributed to leukemic cell infiltration into either bone marrow or parenchymal organs that if left untreated is uniformly fatal. Leukemia, like all forms of cancer, is a genetic disease resulting from mutations in proto-oncogenes or tumor suppressor genes that yield a dominant gain or recessive loss of function respectively. Acute leukemia results as a consequence of multiple acquired mutations that accumulate in hematopoietic precursor cells. These mutations can be grouped into three general categories: point mutations, gross chromosomal rearrangements (GCRs), and epigenetic changes. Numerous GCRs have been observed in leukemia biology. These mutations include balanced and unbalanced chromosomal translocations, as well as chromosomal inversions, deletions, and amplifications. Of these GCRs, chromosomal translocations (CTs) have been observed in many cases of acute and/or chronic leukemia. The analysis of these altered genetic events has proven to be especially useful in understanding the biology of hematopoietic malignancies, leading to improved diagnosis and classifications, as well as identification of novel therapeutic targets.

The rare but recurring CT [t(10;11)(p13;q21)] leads to a *CALM-AF10* fusion gene that occurs in a subset of patients with both acute lymphoblastic leukemia (ALL) and acute myeloid leukemia (AML). To assess the role of the *CALM-AF10* fusion gene in leukemic transformation in vivo as a model of human disease, we generated transgenic mice that expressed a *CALM-AF10* fusion gene in hematopoietic tissue. Of the F1 generation transgenic mice observed at least 40-50 per cent developed acute leukemia at a median age of 12 months. Leukemic mice typically had enlarged spleens, invasion of parenchymal organs with malignant cells, and tumors with myeloid markers such as myeloperoxidase, Mac1, and Gr1. Although most leukemias were myeloid, a subset showed lymphoid features, such as CD3 immunoreactivity, or clonal *Tcrb* or *Igh* gene rearrangements. Mice were clinically healthy for the first 9 months of life, and had normal peripheral blood hemograms, but showed impaired thymocyte differentiation, manifested by decreased CD4+/CD8+ cells and increased immature CD4-/CD8- cells in the thymus. Hematopoietic tissues from both clinically healthy as well as leukemic *CALM-AF10* mice showed up-regulation of *Hoxa* cluster genes, particularly *Hoxa9*, suggesting a potential mechanism for impaired differentiation.

The long latency period and incomplete penetrance of the *CALM-AF10* leukemic phenotype suggests that additional genetic events are needed to complement the *CALM-AF10* transgene and complete the process of leukemic transformation. These results are

consistent with the emerging paradigm in leukemia biology that suggests that most, if not all leukemic cells must undergo at least two collaborative events to produce a fully transformed cell. One event typically leads to impaired differentiation and enhanced renewal of stem cells, whereas the second event leads to increased proliferation and/or decreased apoptosis. To identify complementary genetic events that might collaborate with *CALM-AF10* during leukemic transformation, we used retroviral insertional mutagenesis (RIM). In this study, newborn *CALM-AF10* mice were infected with a modified replication competent retrovirus (Mol4070LTR); by 7 months of age, 90% of the transgenic mice developed acute leukemia. Acute leukemia developed more rapidly in *CALM-AF10* infected mice than either wild-type mice infected with retrovirus ($p = 0.004$) or *CALM-AF10* mice not infected with retrovirus ($p=0.037$). The majority of tumors assayed by Southern blotting for viral integration showed clonal to oligoclonal expansion. Transgenic mice infected with retrovirus developed B-cell ALL, biclonal and biphenotypic leukemia, and T-cell ALL, in addition to AML. Ligation-mediated PCR of DNA isolated from leukemic spleens identified potential collaborating genes near the retroviral insertion sites. Two hundred and sixty two unique integrations were identified in 40 *CALM-AF10* mice. Of these, 55 integrations occurred at 20 common insertion sites, including *Zeb2, Mn1, Evi1, Nf1, Ift57, Mpl, Kras, Vav1,* and *Gata1*.

Of the candidate genes identified, *Zeb2* and *Nf1* are of particular interest. *Zeb2* encodes the transcriptional co-repressor Smad-interacting protein 1, which is a member of the TGF beta signaling pathway. Previous studies using RIM identified *Zeb2* insertions only sporadically, never more than once in a single study, and in association with B-cell, T-cell, and myeloid leukemia. In this study, retroviral integrations near *Zeb2* were identified either by PCR or Southern blot analysis in 26% (11/42) of the *CALM-AF10* mice. RIM studies often generate oligoclonal leukemias, with two or more independent leukemias arising in the same mouse. Southern blot analysis demonstrated that the *Zeb2* integration was present in the dominant leukemic clone in most of these mice, and the *Zeb2* transcript was overexpressed compared to wild-type spleen and bone marrow. Most leukemias that arise in *CALM-AF10* mice without retroviral insertions are myeloid 85% (17/20). However, almost all 91% (10/11) of the *CALM-AF10* mice with *Zeb2* insertions developed B-lineage ALL suggesting that expression of *Zeb2* strongly influences the phenotype of *CALM-AF10* leukemias. Additionally, the tumor suppressor gene *Nf1* (*Neurofibromatosis type 1*) identified in this assay, functions as a GTPase activating protein that regulates the activity of ras proteins involved in cellular proliferation, and acts as a tumor suppressor gene in immature myeloid cells. Retroviral integrations into the *Nf1* locus were identified by PCR or Southern blot in 26% (11/42) of the mice in this study. In at least two cases, Southern blot analysis showed loss of the germline allele. The high frequency of *Nf1* integrations identified in the RIM assay suggested that *Ras* pathway activation complemented the *CALM-AF10* transgene.

We have shown here that expression of *CALM-AF10* in murine hematopoietic cells leads to overexpression of *Hoxa* cluster genes, impairs hematopoietic differentiation, and is ultimately leukemogenic. Furthermore, retroviral insertional mutagenesis accelerates the onset of acute leukemia, alters the immunophenotype, and identifies genes that potentially collaborate with the *CALM-AF10* fusion gene in the leukemic transformation process. This transgenic murine model serves as a model system for studying leukemogenesis similar to that observed in people with leukemia.

Mark J. Hoenerhoff, D.V.M., Ph.D., Diplomate, The American College of Veterinary Pathologists

Dr. Hoenerhoff was a graduate fellow in the NCI molecular pathology GPP in partnership with Michigan State University from 2004-2008.

Dr. Hoenerhoff received his DVM from Michigan State University in 1998, and spent three years in small animal private and emergency practice. In 2001, Dr. Hoenerhoff returned to Michigan State University as a resident in Anatomic Pathology in the Department of Pathobiology and Diagnostic Investigation. Following completion of his residency, Dr. Hoenerhoff accomplished board certification in Anatomic Pathology by The American College of Veterinary Pathologists in 2004, while pursuing graduate training in comparative pathology through the Graduate Partnership Program at the National Cancer Institute, Bethesda, MD. He pursued his dissertation research in cancer biology and genetically engineered mouse models of breast cancer in the Transgenic Oncogenesis Laboratory headed by Jeffrey Green, M.D., in the Laboratory of Cancer Biology and Genetics. Mark defended his dissertation research and received his PhD degree from Michigan State University in May of 2008. The title of his Ph.D. dissertation is "BMI1 collaborates with HRAS to promote mammary tumorigenesis and metastasis". Members of his graduate guidance committee included Thomas Mullaney, D.V.M. Ph.D., (Chair), Kurt Williams, D.V.M., Ph.D., P.S. MohanKumar, B.V.Sc., Ph.D, Michael Scott, D.V.M., Ph.D., Vilma Yuzbasiyan-Gurkan, Ph.D., Jeff Green, M.D., and Mark Simpson, D.V.M., Ph.D. Dr. Hoenerhoff is now a staff pathologist at the National Institute of Environmental Health Sciences in the Cellular and Molecular Pathology Branch, Research Triangle Park, NC.

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Scott Fitzgerald, D.V.M., Ph.D.
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Graduate Fellows
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Heather Tillman, D.V.M.

John Cullen, V.M.D., Ph.D.
Professor, North Carolina State University

Keith Linder, D.V.M., Ph.D.
Assistant Professor, North Carolina State University

Graduate Fellows
Sally Davis, D.V.M.
Leah Zadrozny, D.V.M.

Symposium Participants

Comparative Biomedical Scientist Training Program

Natcher Center, Bethesda, Maryland
October 2–3, 2008

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